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4 **Lipophilic and hydrophilic antioxidants, lipid peroxidation inhibition**  
5 **and radical scavenging activity of two Lamiaceae food plants**  
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17 **Running title:** Lipophilic and hydrophilic antioxidants of Lamiaceae

## ABSTRACT

Medicinal and aromatic plants are highly prized all over the world. According to local cuisine and pharmacopoeias, they used to be important as dietary supplements, providing bioactive compounds. Herein we describe lipophilic (fatty acids, tocopherols and carotenoids) and hydrophilic (ascorbic acid, sugars and phenolic compounds) antioxidants, lipid peroxidation inhibition and free radical scavenging activity in aerial parts of two Lamiaceae species (*Mentha pulegium* and *Thymus pulegioides*). *M. pulegium* gave the highest antioxidant properties ( $EC_{50} < 0.56$  mg/ml), which is in agreement with its highest content in tocopherols, mainly  $\alpha$ -tocopherol (69.54 mg/100 g), ascorbic acid (7.90 mg/100 g), reducing sugars (7.99 g/100 g) and phenolics. The presence of these lipophilic and hydrophilic antioxidants could explain its use as antiseptic, anti-inflammatory and as food preservative and special sauce. *M. pulegium* revealed the highest content of fat,  $\alpha$ -linolenic (omega-3) and linoleic (omega-6) fatty acids, while *T. pulegioides* revealed the highest content of carbohydrates (89.35 g/100 g). This could explain its use to improve the nutrition value of rye flour broth or potato based soups.

**Keywords:** *Lamiaceae*; *Mentha pulegium*/*Thymus pulegioides*; Lipophilic/hydrophilic antioxidants; Lipid peroxidation inhibition; Radical scavenging activity

## 1 Introduction

Even though wild edible species were very important in traditional rural societies current research still appears to be focused on the popular or commonly used species, some of which may have already been fully or partially domesticated. Therefore, it is vital that more research is conducted on potentially exploitable wild species. This would promote their increased utilization thereby simultaneously contributing to conserving their genetic resources [1]. Plants are good sources of natural preparations containing effective bioactive compounds, including lipophilic antioxidants such as tocopherols, carotenoids and unsaturated fatty acids, and hydrophilic antioxidants such as polyphenols and reducing sugars, which can be used for different applications, particularly as food additives and health promoting ingredients in the formulations of functional foods and nutraceuticals [2]. In our continuous study to find new natural antioxidant sources, we focused on two wild Lamiaceae species widely used in two regions of Portugal, Alentejo and Trás-os-Montes. Pennyroyal (*Mentha pulegium* L., port. poejo, manjerico-do-rio) is an emblematic flavour of the gastronomy from Alentejo (Southern Portugal) [3] and also particularly appreciated in Trás-os-Montes (Northeastern Portugal) [4,5]. Very popular all over the country because of the famous liqueur prepared with inflorescences (licor de poejo), the traditional use of the species concerns food and pharmaceutical applications, without a clear frontier between these two purposes. Aerial parts and inflorescences are gathered during summer, dried in shadow and kept at home for seasoning and preparing homemade remedies (infusions, syrups, elixir) recommended for indigestion, stomachache, headache, respiratory system and cholesterol [5,6]. The liqueur and the flower infusion are drunk both for pleasure and for their digestive and carminative properties [4,5]. In Alentejo, a kind of “pesto”, locally known as “piso”, is prepared with fresh plant material, salt, garlic and olive oil, the mix being preserved for future use along the year, when the plant is not

available [3,6,7]. Different recipes of “piso” flavour a very typical cuisine based on fish, bread and different kinds of goat or sheep cheese. Besides, in Alentejo, pennyroyal is usually cultivated nearby the windows to repel flies and mosquitoes in summer [6].

Large thyme, also known as broad-leaved thyme (*Thymus pulegioides* L., port. Pojinha) is a species from the meadows of the north of Portugal, occurring rarely in the southern region. In Trás-os Montes large thyme is highly prized in folk therapy [4]. The species has been traditionally used for its antiseptic and anti-inflammatory properties in the treatment of cold, cough, sinusitis, bronchitis, pneumonia and tuberculosis [4,8]. Carminative and emmenagogue effects have also been reported [4]. Medicinal infusions and tisanes are prepared with the aerial part of the plant, gathered in June and hung in branches to dry. Dried flower heads were often used for seasoning insipid and famine food during starvation periods. Several informants claimed that, besides the nice taste, large thyme also improved the nutritional value of such food, as well as other aromatic herbs, traditionally gathered from the wild [4,5].

Most of the described uses remain undocumented and unstudied from an ethnobotanical and pharmacological perspective. The present work evaluates lipophilic (fatty acids, tocopherols and carotenoids) and hydrophilic (ascorbic acid, sugars and phenolic compounds) antioxidants in aerial parts of two Lamiaceae species (*Mentha pulegium* and *Thymus pulegioides*). Furthermore, it describes their lipid peroxidation inhibition and free radical scavenging activity.

## **2 Materials and methods**

### **2.1 Plant material**

The aerial parts of the two species (inflorescences and leafy flowering stems about 20cm long) were gathered along 2009 summer, in the Natural Park of Montesinho territory, Trás-

os-Montes, North-eastern Portugal, according local folk criteria of use and plant growth patterns. Morphological key characters from the Flora Iberica were used for plant identification. Voucher specimens are kept in the herbarium of *Escola Superior Agrária de Bragança* (BRESA). Each sample was lyophilized (Ly-8-FM-ULE, Snijders, HOLLAND) and kept in the best conditions for subsequent use.

## 2.2 Standards and reagents

Acetonitrile 99.9%, *n*-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Lab-Scan (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers, ascorbic acid, tocopherols and sugars standards, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid and (+)-catechin. Racemic tocol, 50 mg/ml, was purchased from Matreya (PA, USA). DPPH (2,2-diphenyl-1-picrylhydrazyl) was obtained from Alfa Aesar (Ward Hill, MA, USA). All other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

## 2.3. Nutritional value

The samples were analysed for macronutrients composition (moisture, fat, protein and ash) using AOAC procedures [9]. The crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the crude protein content ( $N \times 6.25$ ) of the samples was estimated by the macro-Kjeldahl method; the ash content was determined by incineration at  $600 \pm 15$  °C. Total carbohydrates were calculated by difference. Reducing sugars were determined by DNS (dinitrosalicylic acid) method.

Total energy was calculated according to the following equation: Energy (kcal) = 4 × (g protein + g carbohydrates) + 9 × (g fat).

## 2.4. Lipophilic compounds

Fatty acids were determined by GC/FID (Gas chromatography/Flame ionization detector) as described previously by the same authors [10]. The equipment was a DANI model GC 1000 with a split/splitless injector, a FID and a Macherey-Nagel column (30 m × 0.32 mm ID × 0.25 µm  $d_f$ ). The oven temperature program was as follows: the initial temperature of the column was 50 °C, held for 2 min, then a 10°C/min ramp to 240 °C and held for 11 min. The carrier gas (hydrogen) flow-rate was 4.0 mL/min (0.61 bar), measured at 50 °C. Split injection (1:40) was carried out at 250 °C. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using CSW 1.7 software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

Carotenoids were determined according to Barros et al. [11]. Contents of β-carotene and lycopene were calculated according to the following equations: lycopene (mg/100 mL) =  $0.0458 \times A_{663} + 0.204 \times A_{645} + 0.372 \times A_{505} - 0.0806 \times A_{453}$ ; β-carotene (mg/100 mL) =  $0.216 \times A_{663} - 1.220 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$ . The results were expressed as mg of carotenoids per 100 g of dry weight (dw).

Tocopherols content was determined by HPLC (high-performance liquid chromatography)/fluorescence following a procedure described by us [11], using tocol as internal standard. The equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco) and a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The chromatographic separation was achieved with a

Polyamide II (250 × 4.6 mm) normal-phase column from YMC Waters operating at 30°C. The mobile phase used was a mixture of *n*-hexane and ethyl acetate (7:3, v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response, using the internal standard method. Tocopherol contents in the samples are expressed in mg per 100 g of dry weight (dw).

## 2.5. Hydrophilic compounds

Ascorbic acid was determined according to Barros et al. [11]. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (0.006-0.1 mg/mL;  $y = 3.0062x + 0.007$ ;  $R^2 = 0.9999$ ), and the results were expressed as mg per 100 g of dry weight (dw).

Free sugars were determined by HPLC/RI (Refraction index detector) as described by Heleno et al. [10], using melezitose as internal standard. The equipment described above was coupled to a RI detector (Knauer Smartline 2300). Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH<sub>2</sub> column (4.6 × 250 mm, 5 mm, Knauer) operating at 30°C (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 7:3 (v/v) at a flow rate of 1 mL/min. Sugar identification was made by comparing the relative retention times of sample peaks with standards. Quantification was made by internal normalization of the chromatographic peak area and the results are expressed in g per 100 g of dry weight (dw).

## 2.6 Lipid peroxidation inhibition and radical scavenging activity assays

A fine dried powder (20 mesh; ~1g) was extracted by stirring with 30 mL of methanol at 25 °C at 150 rpm for 1 h and filtered through Whatman No. 4 paper. The residue was then

extracted with one additional 30 mL portion of methanol. The combined methanolic  
 extracts were evaporated at 35°C under reduced pressure (rotary evaporator Büchi R-210),  
 re-dissolved in methanol at a concentration of 10 mg/mL, and stored at 4 °C for further use.  
 Total phenolics and flavonoids were estimated based on procedures described by the  
 authors [11]. Gallic acid (0.05-0.8 mM;  $y = 1.9799x + 0.0299$ ;  $R^2 = 0.9997$ ) and (+)-  
 catechin (0.0156-1.0 mM;  $y = 0.9186x - 0.0003$ ;  $R^2 = 0.9999$ ) were used to calculate the  
 standard curves. The results were expressed as mg of gallic acid equivalents (GAE) and  
 mg of (+)-catechin equivalents (CE), respectively for phenolics and flavonoids, per g of  
 extract. Flavanols were estimated based on the procedure described by Mazza et al. [12].  
 Quercetin (0.2-3.2 mM;  $y = 0.1962x - 0.0636$ ;  $R^2 = 0.9986$ ) was used to calculate the  
 standard curve, and the results were expressed as mg of quercetin equivalents (QE) per g of  
 extract.

The antioxidant activity was evaluated by inhibition of  $\beta$ -carotene bleaching in the  
 presence of linoleic acid radicals, inhibition of lipid peroxidation in brain homogenates by  
 TBARS (thiobarbituric acid reactive substances), reducing power and DPPH radical-  
 scavenging activity assays, following procedures described previously by the authors [11].  
 $\beta$ -Carotene bleaching inhibition was calculated using the following equation: ( $\beta$ -carotene  
 content after 2h of assay/initial  $\beta$ -carotene content)  $\times$  100. The TBARS formation  
 inhibition (%) was calculated using the following formula: Inhibition (%) =  $[(A - B)/A] \times$   
 100%, where A and B were the absorbance of the control and the compound solution,  
 respectively. The radical scavenging activity (RSA) was calculated as a percentage of  
 DPPH discolouration using the equation: % RSA =  $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$ , where  $A_S$  is  
 the absorbance of the solution when the sample extract has been added at a particular level,  
 and  $A_{DPPH}$  is the absorbance of the DPPH solution. The extract concentrations providing  
 50% of antioxidant activity ( $EC_{50}$ ) were calculated from the graphs of antioxidant activity



percentages against extract concentrations (for each assay). Trolox was used as standard.

## 2.7 Statistical analysis

All the assays were carried out in triplicate. The results were expressed as mean values and standard deviation (SD), and were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with  $\alpha = 0.05$  (SPSS v. 16.0 program).

## 3 Results and discussion

In former times, both species were highly consumed and frequently gathered from the wild. Nowadays *M. pulegium* (pennyroyal) is still a very popular species but *T. pulegioides* (large thyme) is less used because special skills are needed to find it feral. As edibles it is important to know and to characterize its nutritional value. The macronutrients profile and estimation of energy content are shown in **Table 1**. Pennyroyal revealed the highest content of moisture, fat, protein, ash and energy, while large thyme revealed the highest content of carbohydrates. These compounds were the most abundant macronutrients.

The results of fatty acid composition, SFA (Saturated Fatty Acids), MUFA (Monounsaturated Fatty Acids) and PUFA (Polyunsaturated Fatty Acids) are shown in **Table 2**. The most abundant fatty acids were  $\alpha$ -linolenic (C18:3), linoleic (C18:2) and palmitic (C16:0) acids (**Figure 2**). Eighteen more fatty acids were identified and quantified, being PUFA the main group. The omega-3 (including  $\alpha$ -linolenic acid) and omega-6 (including linoleic acid) fatty acids are associated with several beneficial health effects in inflammatory diseases, hypertension, heart disease, prostate and breast cancers, among others [13,14]. Furthermore, the ratios PUFA/SFA were higher than 0.45 and the ratios n-6/n-3 fatty acids were lower than 4.0, which are recommended for the human diet,

and contribute to the decrease of total amount of fat in blood (cholesterol), reducing the risk of cancer, cardiovascular, inflammatory and autoimmune diseases [15].

Non-enzymatic antioxidants including lipophilic (carotene and  $\alpha$ -tocopherol) and hydrophilic (ascorbic acid) compounds may play an important role in the cellular response to oxidative stress by reducing certain ROS, and retard the progress of many chronic diseases as well as the lipid oxidative rancidity in food, cosmetics and pharmaceutical materials [16]. The studied plants revealed the presence of those antioxidant molecules (**Table 3**); pennyroyal showed the highest levels of tocopherols, particularly  $\alpha$ -tocopherol (69.54 mg/100 g dw), and ascorbic acid (7.90 mg/100 g), while large thyme gave the highest levels of carotenoids (2.04 mg/100 g).

Other hydrophilic molecules such as sugars were determined and the results are given in **Table 4**. Pennyroyal revealed the highest content of total sugars, and in particular fructose, glucose, sucrose and trehalose, while large thyme revealed the highest content of raffinose. Fructose, glucose, trehalose, raffinose and sucrose were detected in both samples; sucrose was the most abundant sugar. Sucrose and threalose are non-reducing sugars and, therefore, total sugars obtained by HPLC/RI (**Table 4**) were higher than reducing sugars, measured by DNS colorimetric assay (**Table 1**). Sugars were a small part of carbohydrates due to the presence of polysaccharides such as starch and cellulose.

The pharmacological effect of polyphenols (a diverse class of natural products suggested to be the key bioactives present in plant foods), are attributed to their antioxidant (e.g. ROS scavenging activity), indirect antioxidant (e.g. enzyme inhibition), anti-inflammatory as well as gene expression-modifying effects [17]. The amount of phenolics found in wild pennyroyal methanolic extract (331.69 mg GAE/g) was higher than the amount found in other Portuguese sample bought in a traditional market (ethanolic extract 71.7 mg/g; aqueous extract 57.9 mg/g) [18]. Otherwise the amount found in large thyme methanolic

extract (210.49 mg/g) was lower than the amount found in ethanolic extract from Italian material (435.1 mg/g) [17]. These molecules were also found in *Thymus pulegioides* chemotypes from Lithuania [2] and in Algerian samples [19]. As it can be observed in **Table 4**, flavanols represent an important group of flavonoids in both spices.

Pennyroyal and large thyme methanolic extracts showed antioxidant properties measured by four different assays targeted to lipid peroxidation inhibition and radical scavenging activity evaluation (**Table 4**). Pennyroyal gave the highest antioxidant activity ( $EC_{50}$  values  $< 0.56$  mg/ml), may be due to its major content in hydrophilic antioxidants (phenolics, flavonoids, ascorbic acid and reducing sugars) but also in lipophilic antioxidants such as tocopherols. Other authors also correlated the antioxidant activity found in *Thymus pulegioides* from Lithuania [2] and in *Mentha pulegium* from Spain [20] with the concentration of polyphenolic compounds such as phenolic acids and flavonoids. The antioxidant activity of large thyme ethanolic extract from Italy [17] and of pennyroyal methanolic extract from Spain [21] was also reported. A pennyroyal sample bought in a Portuguese traditional market gave higher DPPH radical scavenging activity ( $EC_{50}$  value for ethanolic extract: 24.9  $\mu$ g/ml), but lower  $\beta$ -carotene bleaching inhibition capacity (165  $\mu$ g/ml) [18] than our sample (**Table 4**).

The studied plants could have some potential in food industry because of its flavouring properties and nutritional composition (including omega-3 and omega-6 fatty acids, and sugars), but also in pharmaceutical industry due to its biological and medicinal benefits (demonstrated by their *in vitro* lipid peroxidation inhibition and radical scavenging properties). The presence of lipophilic (carotenoids and tocopherols) and hydrophilic (ascorbic acid, phenolics and flavonoids) antioxidants, could be related to their traditional uses as antiseptic and anti-inflammatory (activities related to oxidative stress) [4,8,22].

Furthermore, the presence of those compounds could explain the use of pennyroyal as food preservative. The nutritional value of large thyme characterized by high levels of carbohydrates could also explain its use to improve the nutrition value of some food usually eaten long ago during famine periods, such as potato based soups seasoned with the leaves and a broth prepared with the top leaves and flowers boiled in water and then thickened with a tablespoon of rye [4,5,18].

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## Conflict of interest statement

The authors have declared no conflict of interest.

## References

- [1] M. V. Flyman, A. J. Afolayan: The suitability of wild vegetables for alleviating human dietary deficiencies. *South Afr J Bot.* 2006, 72, 492-497.
- [2] K. Loziene, P. R. Venskutonis, A. Sipailiene, J. Labokas: Radical scavenging and antibacterial properties of the extracts from different *Thymus pulegioides* L. chemotypes. *Food Chem.* 2007, 103, 546-559.
- [3] O. Póvoa: Produção e utilização dos taxa *Mentha pulegium* L. e *Mentha cervina* L., PhD Thesis, Universidade Técnica de Lisboa 2008.
- [4] A. M. Carvalho: Etnobotánica del Parque Natural Montesinho. Plantas, tradición y saber popular en un territorio del nordeste de Portugal, PhD Thesis, Universidad Autónoma de Madrid 2005.

- [5] M. P. Santayana, J. Tardio, E. Blanco, A. M. Carvalho, J. J. Lastra, E. San Miguel, R. Morales: Traditional knowledge of wild edible plants used in the northwest of the Iberian Peninsula (Spain and Portugal): a comparative study. *J Ethnobiol Ethnomed* 2007, 3, 27-37.
- [6] O. Póvoa, S. Marinho, N. Farinha: Preliminary study of hart's pennyroyal and pennyroyal ethnobotany in Alentejo, Proceedings of the 9<sup>th</sup> International Congress of Ethnobiology, University of Kent, Canterbury 2004.
- [7] O. Póvoa, G. Ribeiro, L. Rodrigues, P. Lobato, P. Monteiro, A. Monteiro, M. Moldão-Martins: Chemical characterization and organoleptical evaluation of a *M. pulegium* L. and *M. cervina* L. «piso», a traditional food sauce. *Acta Horticult.* 2009, 826, 193-200.
- [8] A. P. Cunha, J. A. Ribeiro, O. R. Roque: Plantas aromáticas em Portugal. Caracterização e utilizações. Lisboa: Fundação Calouste Gulbenkian 2007.
- [9] AOAC: Official methods of analysis (16<sup>th</sup> Ed.). Arlington VA, USA: Association of Official Analytical Chemists 1995.
- [10] S. A. Heleno, L. Barros, M.J. Sousa, A. Martins, I. C. F. R. Ferreira: Study and characterization of selected nutrients in wild mushrooms from Portugal by gas chromatography and high performance liquid chromatography. *Microchem J.* 2009, 93, 195–199.
- [11] L. Barros, S. A. Heleno, A. M. Carvalho, I. C. F. R. Ferreira: Systematic evaluation of the antioxidant potential of different parts of *Foeniculum vulgare* Mill. from Portugal. *Food Chem Toxicol.* 2009, 47, 2458-2464.
- [12] G. Mazza, L. Fukumoto, P. Delaquis, B. Girard, B. Ewert: Anthocyanins, phenolics, and color of Cabernet Fran, Merlot, and Pinot noir wines from British Columbia. *J Agric Food Chem.* 1999, 47, 4009-4017.

- [13] P. D. Terry, J. B. Terry, T. E. Rohan: Long-chain (n-3) fatty acid intake and risk of cancers of the breast and prostate: recent epidemiological studies, biological mechanisms, and directions for future research. *J Nutr.* 2004, 134, 3412S–3420S.
- [14] L. Djousse, D.K. Arnett, J. S. Pankow, P. N. Hopkins, M. A. Province, R. C. Ellison: Dietary linolenic acid is associated with a lower prevalence of hypertension in the NHLBI family heart study. *Hypertension* 2005, 45, 368-373.
- [15] J. L. Guil, M. E. Torija, J. J. Giménez, I. Rodriguez. Identification of fatty acids in edible wild plants by gas chromatography. *J. Chromatogr. A* 1996, 719, 229-235.
- [16] V. Lagouri, E. Nisteropoulou: Free radical scavenging and ferric reducing antioxidant properties of *O. onites*, *T. vulgaris* and *O. basilicum* from Greece and their rosmarinic acid content. *Free Rad Res.* 2009, 43, S27-97.
- [17] The Local Food-Nutraceuticals Consortium: Understanding local Mediterranean diets: a multidisciplinary pharmacological and ethnobotanical approach. *Pharmacological Res.* 2005, 52, 353-366.
- [18] A. T. Mata, C. Proença, A. R. Ferreira, M. L. M. Serralheiro, J. M. F. Nogueira, M. E. M. Araújo: Antioxidant and antiacetylcholinesterase activities of five plants used as Portuguese food spices. *Food Chem.* 2007, 103, 778-786.
- [19] P. Stocker, M. Yousfi, O. Djerridane, J. Perrier, R. Amziani, S. E. I. Boustani, A. Moulin: Effect of flavonoids from various Mediterranean plants on enzymatic activity of intestinal carboxylesterase. *Biochimie* 2004, 86, 919-925.
- [20] V. López, S. Akerreta, E. Casanova, J. M. García-Mina, R. Y. Cavero, M. I. Calvo: *In vitro* antioxidant and anti-rhizopus activities of Lamiaceae herbal extracts. *Plant Food Hum Nutr.* 2007, 62, 151-155.

- 332 [21] V. López, S. Martín, M. P. Gómez-Serranillos, M. E. Carretero, A. K. Jäger, M. I.  
333 Calvo: Neuroprotective and neurochemical properties of mint extracts. *Phytother*  
334 *Res.* 2010, 24, 869-874.
- 335 [22] S. Franova, G. Nosalova, J. Mokry: Phytotherapy of cough. *Adv Phytomed.* 2006, 2,  
336 111-131.

**Table 1.** Nutritional value of two Lamiaceae (mean  $\pm$  SD; n=3). In each row, different letters mean significant differences ( $p<0.05$ ).

	<i>Mentha pulegium</i>	<i>Thymus pulegioides</i>
Moisture (g/100 g fw)	59.47 $\pm$ 9.22 a	47.66 $\pm$ 12.60 a
Fat (g/100 g dw)	2.22 $\pm$ 0.22 a	0.18 $\pm$ 0.02 b
Proteins (g/100 g dw)	7.12 $\pm$ 0.49 a	5.53 $\pm$ 1.40 b
Ash (g/100 g dw)	5.92 $\pm$ 0.09 a	4.94 $\pm$ 0.62 b
Carbohydrates (g/100 g dw)	84.74 $\pm$ 0.59 b	89.35 $\pm$ 1.54 a
Energy (Kcal/100 g dw)	387.44 $\pm$ 0.53 a	381.14 $\pm$ 1.76 b
Reducing Sugars (g/100 g dw)	7.99 $\pm$ 1.72 a	2.11 $\pm$ 0.15 b



**Table 2.** Composition in fatty acids (percentages) of two Lamiaceae (mean  $\pm$  SD; n=3). In each row different letters mean significant differences ( $p < 0.05$ ).

	<i>Mentha pulegium</i>	<i>Thymus pulegioides</i>
C6:0	0.79 $\pm$ 0.04	0.28 $\pm$ 0.05
C8:0	1.03 $\pm$ 0.06	nd
C12:0	1.75 $\pm$ 0.20	0.24 $\pm$ 0.01
C14:0	3.42 $\pm$ 0.07	5.70 $\pm$ 0.74
C14:1	0.32 $\pm$ 0.02	1.42 $\pm$ 0.16
C15:0	0.14 $\pm$ 0.04	0.30 $\pm$ 0.00
C16:0	14.82 $\pm$ 0.09	16.70 $\pm$ 0.22
C16:1	0.11 $\pm$ 0.01	nd
C17:0	0.52 $\pm$ 0.07	nd
C18:0	4.96 $\pm$ 0.03	3.39 $\pm$ 0.05
C18:1n9	5.77 $\pm$ 0.20	11.40 $\pm$ 0.10
C18:2n6	16.27 $\pm$ 0.33	12.98 $\pm$ 0.52
C18:3n3	37.00 $\pm$ 0.35	36.69 $\pm$ 0.25
C20:0	2.36 $\pm$ 0.07	1.37 $\pm$ 0.08
C20:1	0.63 $\pm$ 0.02	nd
C20:2	0.15 $\pm$ 0.01	nd
C20:3n3 and C21:0	0.20 $\pm$ 0.04	nd
C22:0	0.90 $\pm$ 0.07	nd
C22:2	1.93 $\pm$ 0.09	nd
C23:0	5.46 $\pm$ 0.05	8.04 $\pm$ 0.41
C24:0	1.47 $\pm$ 0.27	1.50 $\pm$ 0.18
SFA	37.62 $\pm$ 0.83 a	37.52 $\pm$ 0.82 a
MUFA	6.82 $\pm$ 0.19 b	12.81 $\pm$ 0.05 a
PUFA	55.48 $\pm$ 0.53 a	49.67 $\pm$ 0.77 b
PUFA/SFA	1.48 $\pm$ 0.05 a	1.32 $\pm$ 0.05 b
n-6/n-3	0.44 $\pm$ 0.00 a	0.35 $\pm$ 0.01 b

C6:0 (caproic acid); C8:0 (caprylic acid); C12:0 (lauric acid); C14:0 (myristic acid); C14:1 (myristoleic acid); C15:0 (pentadecanoic acid); C16:0 (palmitic acid); C16:1 (palmitoleic acid); C17:0 (heptadecanoic acid); C18:0 (stearic acid); C18:1n9 (oleic acid); C18:2n6 (linoleic acid); C18:3n3 ( $\alpha$ -linolenic acid); C20:0 (arachidic acid); C20:1 (eicosenoic acid); C20:2 (*cis*-11,14-eicosadienoic acid); C20:3n3 and C21:0 (*cis*-11, 14, 17-eicosatrienoic acid and heneicosanoic acid); C22:0 (behenic acid); C22:2 (*cis*-13,16-docosadienoic acid); C23:0 (tricosanoic acid); C24:0 (lignoceric acid); SFA (Saturated Fatty Acids); MUFA (Monounsaturated Fatty Acids); PUFA (Polyunsaturated Fatty Acids); nd- not detected.

**Table 3.** Composition in carotenoids and vitamins of two Lamiaceae (mean  $\pm$  SD; n=3). In each row different letters mean significant differences ( $p<0.05$ ).

	<i>Mentha pulegium</i>	<i>Thymus pulegioides</i>
Carotenoids (mg/100 g dw)	0.42 $\pm$ 0.00 b	2.04 $\pm$ 0.04 c
$\alpha$ -tocopherol	69.54 $\pm$ 11.44 a	12.63 $\pm$ 0.82 b
$\beta$ -tocopherol	1.84 $\pm$ 0.26 a	0.08 $\pm$ 0.00 b
$\gamma$ -tocopherol	9.84 $\pm$ 1.54 a	0.77 $\pm$ 0.08 b
$\delta$ -tocopherol	8.48 $\pm$ 1.55 a	nd
Total tocopherols (mg/100 g dw)	89.70 $\pm$ 14.79 a	13.48 $\pm$ 0.91 b
Ascorbic acid (mg/100 g of dw)	7.90 $\pm$ 0.17 a	5.95 $\pm$ 0.11 b

nd- not detected

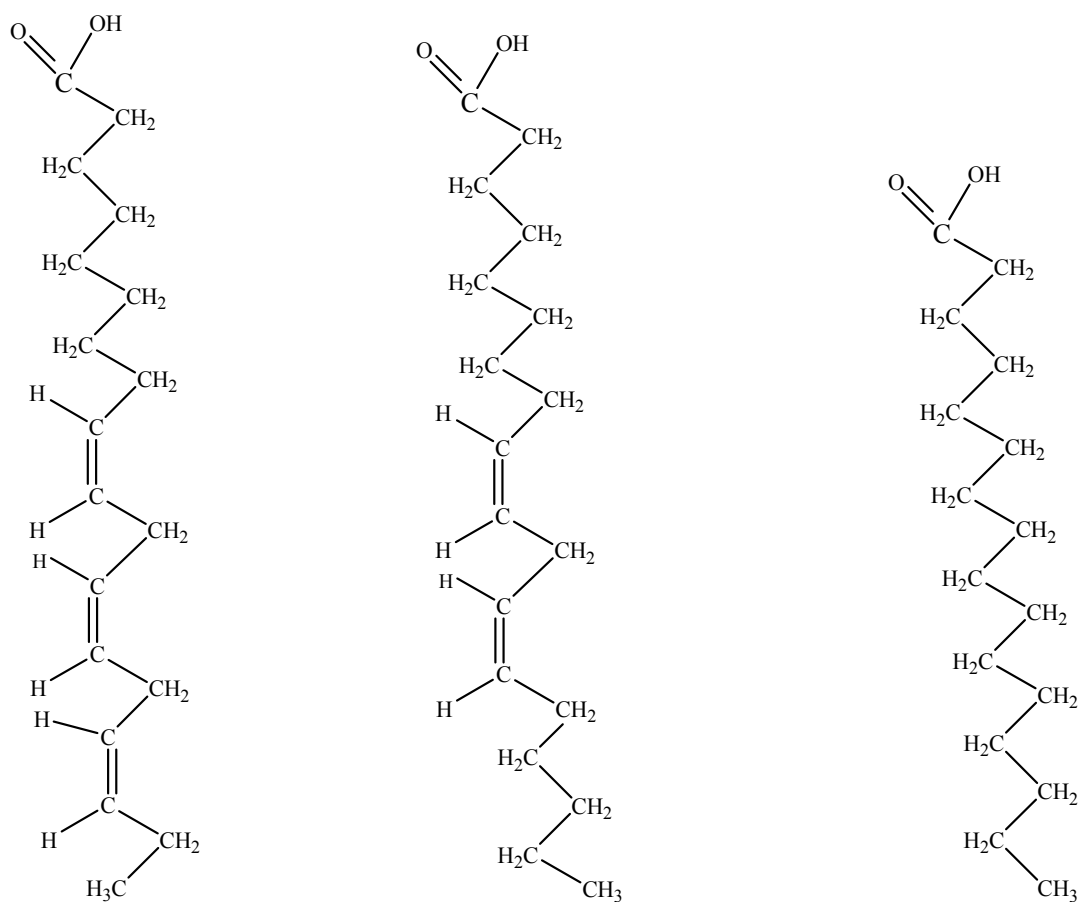
**Table 4.** Composition in sugars of two Lamiaceae (mean  $\pm$  SD; n=3). In each row, different letters mean significant differences ( $p<0.05$ ).

	<i>Mentha pulegium</i>	<i>Thymus pulegioides</i>
Fructose	2.39 $\pm$ 0.11 a	0.22 $\pm$ 0.00 b
Glucose	3.37 $\pm$ 0.22 a	0.33 $\pm$ 0.03 b
Sucrose	4.62 $\pm$ 0.28 a	1.06 $\pm$ 0.02 b
Trehalose	0.61 $\pm$ 0.05 a	0.24 $\pm$ 0.04 b
Raffinose	0.29 $\pm$ 0.05 b	0.55 $\pm$ 0.04 a
Total Sugars (g/100 g dw)	11.29 $\pm$ 0.61 a	2.39 $\pm$ 0.13 b

**Table 5.** Extraction yields, composition in phenolics and flavonoids, and antioxidant activity EC<sub>50</sub> values of two Lamiaceae (mean  $\pm$  SD; n=3). In each row different letters mean significant differences ( $p<0.05$ ).

	<i>Mentha pulegium</i>	<i>Thymus pulegioides</i>
$\eta$ (%)	54.62 $\pm$ 4.26 a	24.61 $\pm$ 0.60 b
Phenolics (mg GAE/g extract)	331.69 $\pm$ 19.63 a	210.49 $\pm$ 21.16 b
Flavonoids (mg CE/g extract)	139.85 $\pm$ 1.27 a	128.24 $\pm$ 6.00 b
Flavanols (mg QE/g extract)	128.57 $\pm$ 0.62 a	126.74 $\pm$ 0.59 a
$\beta$ -carotene bleaching inhibition (mg/mL)	0.01 $\pm$ 0.00 b	0.03 $\pm$ 0.00 a
TBARS inhibition (mg/mL)	0.08 $\pm$ 0.00 b	0.22 $\pm$ 0.01 a
Reducing power (mg/mL)	0.12 $\pm$ 0.01 b	0.49 $\pm$ 0.03 a
DPPH scavenging activity (mg/mL)	0.56 $\pm$ 0.05 b	0.68 $\pm$ 0.03 a

**Figure 1.** Aerial parts with inflorescences of *Mentha pulegium*, a culinary herb, folk remedy and insecticide, and *Thymus pulegioides*, widely reputed as antiseptic and anti-inflammatory. These two Lamiaceae are traditionally used in Portugal.



**Figure 2.** Chemical structures of  $\alpha$ -linolenic, linoleic and palmitic acids.